FSUM 10302
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Robert A. Holton et al.
Serial No. 09/063,477
Filed April 20, 1998
For PROCESS FOR THE SELECTIVE DERIVATIZATION OF TAXANES
Examiner Ba K. Trinh

TO THE COMMISSIONER OF PATENTS AND TRADEMARKS

SIR:

DECLARATION OF DAVID J. PROCTER UNDER 37 C.F.R. 1.608(b)

I, David J. Procter, declare and state as follows:

1. At the time of the invention, I was a post doctorate student at Florida State University in Tallahassee, Florida, where I conducted research in Dr. Robert Holton's laboratory in the area of Synthetic Organic, Biorganic, and Organometallic Chemistry.

2. I am not an inventor of "Process For The Selective Derivatization Of Taxanes" of application no. 09/063,477.

3. During my tenure at Florida State University, I worked in the same laboratory as Zhuming Zhang. Zhuming Zhang worked in close proximity to me, and we routinely exchanged information about our experimental results. I observed Zhuming Zhang, Paul A. Clarke and Dr. Robert Holton reduce to practice the "Process For The Selective Derivatization Of Taxanes" before May 21, 1997.

4. Before May 21, 1997, I observed Zhuming Zhang conduct the "Attempt to protect C(7)OH by cbz" experiment, the "Attempt to protect C(10) H by (CH_3CO)-O" experiment, and the "Generation of baccatin III from 10DAB" experiment as documented on laboratory notebook pages 45, 49 and 67, respectively (Exhibits A-C). I recall that Zhuming Zhang immediately informed me of the results of these experiments so that I could use these processes to significantly simplify my own experimental research. Once Zhuming Zhang told me of his discoveries, I used these processes in preparing other taxane derivatives. The processes discovered by Zhuming Zhang eliminated about five process steps from my own experimental research in preparing the taxane derivatives.

5. After observing Zhuming Zhang conduct the "Attempt to protect C(10) H by (CH_3CO)-O" experiment shown on his laboratory notebook page 49, I used his process in my own research. For example, I conducted the "Selective Protection of 10-Hydroxyl - Formation of 10-Allyloxycarbonate" experiment shown on laboratory notebook pages 177 and 178 (Exhibit D). I added 0.137 ml of diallylpyrocarbonate to 30 mg of 10-deacetyl baccatin III in tetrahydrofuran solvent at room temperature and allowed the mixture to react while adding additional diallylpyrocarbonate at 21, 23, and 26 hours. The final reaction mixture revealed the formation of 10-allyloxycarbonate in about 63% of the reaction product.

6. After observing Zhuming Zhang conduct the "Generation of baccatin III from 10 DAB" experiment shown on his laboratory notebook page 67, I used his process in my own research. For example, I conducted the "Direct Acetylation of 10-DAB - Prepn. of B-III ($ZnCl_2$, O°C)" experiment shown on laboratory notebook pages 167 and 168 shortly after his discovery (Exhibit E). I added 4 ml of acetic anhydride to 113 mg of 10-deacetyl baccatin III and 0.42 ml $ZnCl_2$ in tetrahydrofuran solvent at room temperature. Upon purification, 94.8 mg baccatin III was recovered, which was about 78% yield.

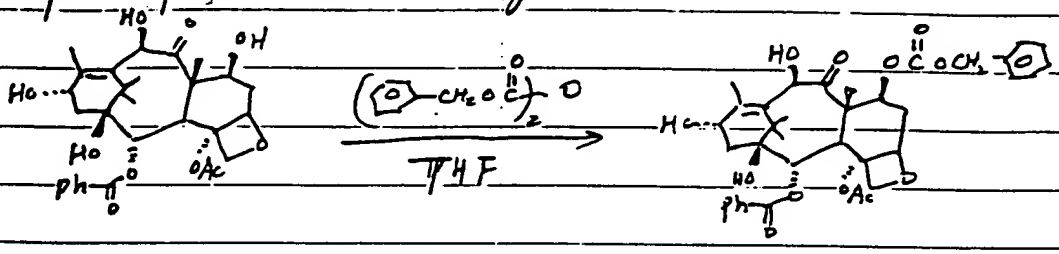
7. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1/8/00

Date

David J. Procter

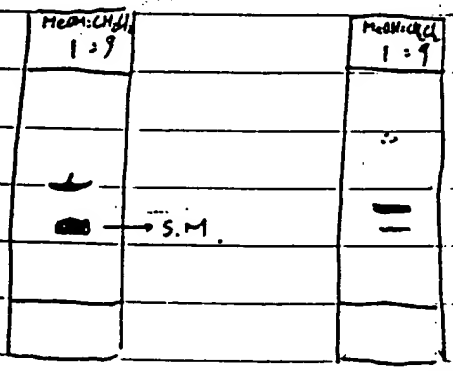
Attempt to protect C(7) OH by Cbz



Materials used	FW	AMT
(1) 10 DAB	544	3 mg (0.00551 mmol)
(2) dibenzyl dicarbonate (97%)	286.29	53 mg (3 eq., 0.484 mmol)
(3) THF		0.5 mL

Procedure: same as Page 44, except THF was used instead of CH_2Cl_2

The rxn proceeded smoothly, TLC indicated the formation of product. The rxn was left for overnight.

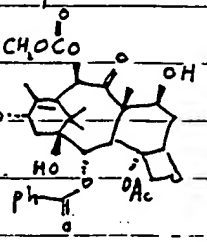


a 5 h overnight.

This rxn almost go to completion, very, very promising !!

^1H NMR @ 2-45-1 mixture of crude

2-45-2
2-45-3
2-45-4

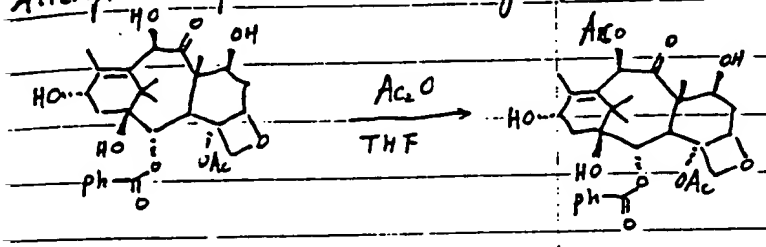


So Cbz attached to C(10) OH

What a surprise ???!

high yield is obtained (≥90%)

Attempt to protect C(10) H by $(CH_3C(=O))_2O$



Materials used	FW	AMT
(1) 10 DAB	544	3 mg (0.00551 mmol)
(2) Ac_2O	102.09	10 μL (20 eqv., 0.11 mmol)
	d = 1.082	
(3) THF		0.5 mL

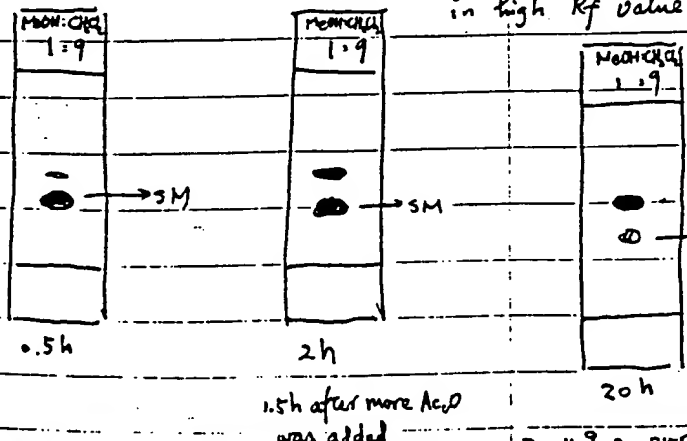
Procedure: the rxn of 10DAB with $(PhCH_2OC(=O))_2O$ suggested that similar reaction could take place between 10DAB + Ac_2O

To a solution of 10DAB in THF was added Ac_2O under nitrogen. The reaction mixture was stirred at room temperature and monitored by TLC. TLC indicated the slow process of the rxn and a faint amount of product formed. At this stage it is not clear whether the rxn is proceeding or not. So 100 μL more Ac_2O was added, After 2h, visible rxn was observed, a single spot shew up in high Rf value. After overnight, ≥80% conversion was observed. At this stage, this rxn was stopped by evaporating the solvent away. and taken by ^1H NMR

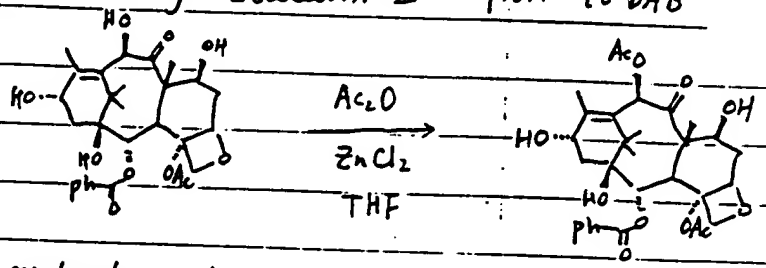
crude ^1H NMR 2-49-1 indicated ≥80% Baccatin III + 10 DAB + small amount of (7-Ac? 10DAB) (by evaporating the solvent?)

2-49-2 pure Baccatin III.

TLC



Generation of baccatin III from 10 DAB

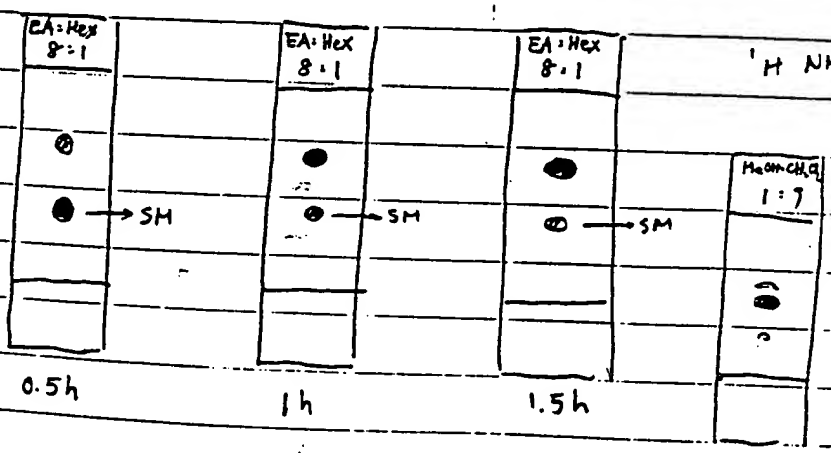


Materials used	FW	AMT
(1) 10 DAB	544	14 mg (0.0258 mmol)
(2) Ac_2O	102.09	1 mL
	d = 1.082	
(3) $ZnCl_2$	136.28	3.5 mg (0.0258 mmol, 1 eq.)
(4) THF		1 mL

Procedure: To a THF solution of 10DAB + $ZnCl_2$ was added Ac_2O under N_2. The solution was stirred at room temperature and monitored by TLC.

actually 18 mg $ZnCl_2$ (may have a little bit of H_2O)

TLC



^1H NMR 2-67-1 crude mixture (too dilute)

2-67-2 crude mixture Major baccatin III + small amount 7-HO-diacetyl + oxetane ring opened product,

Low temperature experiment is recommended.

DIRECT ACETYLATION OF 10-DAB — PREPN OF B-III ($ZnCl_2$, 0°C).

TRANSFORMATION —

QUANTITIES

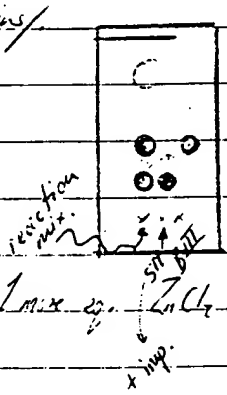
	QUANT:	MOL. WT:	MMOLS:	EQUIV:	SOURCE:
+ slight impurity + d=1.052 — 10-DAB (recovered)	~113mg	544	0.21	1	—
Ac_2O	4ml	″	″	″	″
$ZnCl_2$ (0.5M THF)	0.42ml	″	0.21	1	
THF	2ml	″	″	″	″

METHOD — To the resulting soln added to a soln of 10-DAB (113mg.) in THF (2ml) at 0°C. To Ac_2O (4ml) under N_2 was added $ZnCl_2$ in THF (0.42ml)

RXN. MONITORING — On at 12.24 Fri.

50 mins/ 3h/ - trace of 10-DAB remaining.

4h/ - trace still remaining



80% EA/HEX

UV/Anisaldehyde

+ 1 mix sp. $ZnCl_2$ (0.42ml) added.

W-up — rxn. mix. poured gradually into 45ml ($NaHCO_3$) ~~added~~.

Aq. layer extracted with EtOAc (3×5ml) dried (Na_2SO_4) & concd. in vacuo => DJP1-167-82.0

COMMENTS — Crude looks good ~16% bis acetate (+ trace of oxetane ring-opened product). otherwise clean.

(60 mg. 11 %).

Purification FLASH (50% EA/HEX)

[13-20] => bis acetate

[22-45] => BIII (94.8 mg, ~78 %)

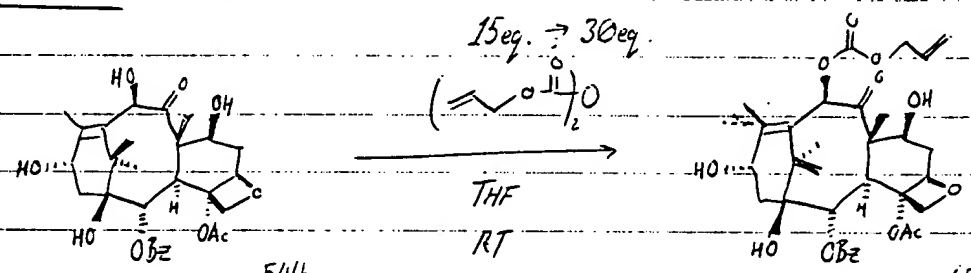
-> DJPI-167-82-1

SELECTIVE PROTECTION OF 10-HYDROXYL

- FORMATION OF 10-ALLYL OXYCHARBONATE

{see ZZ 74}.

TRANSFORMATION



QUANTITIES

		QUANT:	MOL.WT:	MMOLS:	EQUIV:	SOURCE:
	10-DAB	~30mg	544	0.055	1	/
d=1.121	diallyl pyrocarbonate	0.137ml	186.17	0.827	15	ALDRICH
	THF	0.5ml	/	/	/	/

METHOD

To a soln of 10-DAB (30mg) in THF (0.5ml), at RT was added diallyl pyrocarbonate (0.137ml, 15eq) & the rxn mixture stirred at RT for h & the process followed by TLC.

RXN MONITORING

On at 3.15pm Mon.

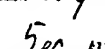
1h/

80% EA/HEX

UV/Anisaldehyde.

2h/ - rxn. proceeding slowly.

16h/ rxn. gone ~50%

21h/ 5eq more of ()$_2$O added.

23h/ THF (0.5ml) & 10eq more reagent added (now identical conditions to ZZ).

26h/ 16eq more added (40eq. in total).

40h/ - rxn. still not complete.

Work up - rxn. mix. added to FLASH col. washed (50% EA/HEX).

RECOV [2-5] => des.prod. + 1E DHB

[6-11] => 1E DHB.

FLASH (40% EA/HEX)

[4-7] => product (virtually clean).

(22 mg. ~63 %) => DJP1-177-87.0

[8-11] => 1E-DHB

(16.4 mg. ~32 %)

NEXT TIME

- vac off TLC

- [illegible] with 10% EA/Hex

- [illegible] EA/Hex

Recovered reagent DJP1-177-87.1

ALDRICH reagent DJP1-177-87.2

recovered completely